UNITED STATES DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

Inventors : Aki Kobayashi et al)
Serial No. : 10/500,358) ART UNIT: 1617
Filing Date : June 30th 2004) EXAMINER:
Mr. Yong S Cchong
Title : Antiseptic disinfectant, and cosmetics and toiletries, medicine or containing the same

Commissioner for Patents
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DECLARATION

1. We, Fumihiro Okada and Hiroya Okamoto, hereby declare as follows.

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(1). I, Fumihiro Okada, was born on February 11th, 1966, and graduated from Chemistry Faculty of Science and engineering, Ritsumeikan University in March, 1989, and earned doctorate in Science as "No. 041 of Osaka Prefecture University".

(2) My backgrounds from the graduation to the present are following.

- 1989, April 1st Research worker in Development Research Laboratories of MANDOM CORPORATION
- 1997, April 1st Section head in Fundament Research Laboratories of MANDOM CORPORATION
- 2002, April 1st to date laboratory head in Quality Estimation Laboratories of MANDOM CORPORATION
- 2004, April 1st to date Affiliate associate professor in Japan Advance Institute of Science And Technology

(3) Societies which I belong to are following.

・1990, April The society for antibacterial and antifungal agents, Japan as a member (as a member of editorial board of the society from 2004, April to date, as a councilor from 2007, January as well)

・1991, April to date Japanese Society for Bacteriology as a member

・1994, April to date The Japanese Biochemical Society as a member

・1999, May to date The Society of Cosmetic Chemists of Japan as a committee member

・2001, May to date Japanese Society for contact dermatitis as a representative member

(4) Papers I presented are following.

(Academic paper)

1. Preservative Evaluation of Cosmetics and Toiletories by Microbial Calorimetry /Okada, F.., Fujiwara, N.., Matsuyama, K., Takahashi, K. /*J. Soc. Cosmet. Chem. Jpn,.,* 27(3), 242-248 (1993)
2. Simplified, Time-saving Microbial Tests for Cosmetics and Toiletories / Okada, F /*J. Soc. Cosmet. Chem. Jpn,.,* 32(2), 131-139 (1998)
3. Quantitative Analysis of Antimicrobial Actions of Drugs Studied by Microbial Calorimetry / Okada, F / *Netsu Sokutei.,* 25(4), 132-137 (1998)
4. Microbial Calorimetry of Supported Cultures and Its Application to the Study of Antimicrobial Action / Okada, F.., Kobayashi, A., Fujiwara, N.., Takahashi, K. / *Biocontrol Science*, 3, pp.79-85 (1998)
5. Bacteriostatic and Bactericidal Actions of Antimicrobial Drugs Studies by Microbial Calorimetry / Okada, F.., Kobayashi, A., Fujiwara, N.., Takahashi, K. / *Biocontrol Science*, 4, pp.35-39 (1999)
6. Calorimetric Analysis of Antimicrobial Effect of *p*-hydroxybenzoic Acid Alkyl Esters / Okada, F.., Kobayashi, A., Fujiwara, N.., Arimoto, N., Takahashi, K. / *Biocontrol Science*, 4, pp.67-73 (1999)
7. Calorimetric Study of the Antimicrobial Action of Various Polyols Used for Cosmetics and Toiletries / Aono, A., Takahashi, K., Mori, N., Shimizu, H., Kobayashi, A., Fujiwara, N.., Okada, F.. / *Netsu Sokutei.,* 26(1), 2-8 (1999)
8. Various materials used in Cosmetics and Toiletries, or medicine, having antibacterial effects / Okamoto, H., Kobayashi, A., Okada, F. / "Course relating to microorganism, and antiseptic agent or disinfecting agent used in cosmetics and toiletries, or medicine" in Journal of The society for

antibacterial and antifungal agents, Japan 28, (12) , 801-810 (2000)

9. Solution to Environmental Pproblems on Formulation Development of Cosmetics and Toiletries / Okada, F., / FRAGRANCE JOURNAL, 5, 47-50 (2003)

(Books)

1. Materials having antiseptic disinfectant effects / Okada, F., / Microbial contamination Protection and Antiseptic Design Engineering for Cosmetics and Toiletries · external preparation, Technical Information Institute Co., Ltd, pp.147-157 (2001)

3.

(1) I, Hiroya Okamoto, was born on November 27th, 1969, and graduated from applied biology department of fiber faculty, Kyoto Institute of Technology in March, 1993.

(2) My backgrounds from the graduation to the present are following.

- 1993, April 1st Research worker in Product Development Research Laboratories of MANDOM CORPORATION
- 2004, April 1st to date Section head in Quality Estimation Laboratories of MANDOM CORPORATION

(3) Papers I presented are following.

1. Various materials used in Cosmetics and Toiletries, or medicine, having antibacterial effects / Okamoto, H., Kobayashi, A., Okada, F, / "Course relating to microorganism, and antiseptic agent or disinfecting agent used in cosmetics and toiletries, or medicine" in Journal of The society for antibacterial and antifungal agents, Japan 28, (12) , 801-810 (2000)
2. Application of 1,2-alkanediol having antibacterial effects on Cosmetics and Toiletries / Okamoto, H. / FRAGRANCE JOURNAL, 4, 34-38 (2006)
3. Current development trend of adiaphoretic and deodorant products for men / Okamoto, H., Endo, Y., Kasahara, K. / FRAGRANCE JOURNAL, 5, 38-42 (2006)

4. We are each one of the inventors of US patent application Serial No. 10/500,358. "Antiseptic disinfectant, and cosmetics and toiletries, medicine or containing the same", and have a full knowledge regarding the contents thereof.

5. Hereinafter, we would like to present the test results obtained from the side by side comparison with the closest prior art.

[Object]

The object is to confirm whether perfumes including Camphor which is described in the McCue et al. and essential oil or extract known as antibacterial compounds enhance the antibacterial activity that 1,2-alkanediol originally has against a broad range of strain by combining those with 1,2-alaknediol.

[Method]

The test was performed according to the method described in US patent application "Serial No. 10/500,358. Thus, the method is as bellow.

(Selection of samples)

Among Perfumes, Farnesol, α-Bisabolol, Limonene and Camphor were selected, and then tested.

Among the essential oil or extract known as having antibacterial activity, Citronella oil, Eucalyptus oil, Basil oil and striped bamboo extract were selected, and then tested.

(Sample bacterias)

Candida albicans IFO1594 (mycotic stomatitis), Staphylococcus aureus IFO13276 (Staphylococcus aureus), and Pseudomonas aeruginosa IFO13275 (Pseudomonas) and Escherichia coli IFO3972 were used as sample bacterias.

(Preparation of bacteria solution)

For preparing bacteria, as to Staphylococcus aureus, Pseudomonas and Escherichia coli, they were incubated at 35 °C in the agar medium, and further incubated at 35 °C. after transferred into the bouillon medium. The inoculating bacteria were prepared by diluting the obtained culture solution to about 10^8 inoculating cell /ml.

As to yeast (Candida albicans), the inoculating bacteria were prepared by incubating the yeast in the same way as Staphylococcus aureus, Pseudomonas and Escherichia coli at 30 °C. and diluting the yeast to about 10^7 cell /ml.

(Preparation of Diluting Series of the Test Material)

With a dilution solvent of ethyl cellosolve of 20 w/w %, 1,2-octanediol

solution of 5, 4, 3, 2.5, 2.25, 2, 1.75, 1.5, 1.25 and 1 w/v % were prepared.

While, as to the samples such as the perfume, essential oil or extract, and as to the 50:50 by weight mixture of 1,2-octanediol and the samples, the dilution series were prepared by doubling diluting the 20w/v % solution.

(Measurement of Minimum Inhibitory Concentration (MIC))

1 ml of each of the above-mentioned dilution series including the test material and 9 ml of the agar medium were introduced to each schales, and the above-mentioned inoculating bacteria was applied on each of the schales at the length of 1 cm.

Staphylococcus aureus, Pseudomonas and Escherichia coli were incubated at 35 °C., and it was judged whether or not the bacteria had grown two days later. The yeast was incubated at 25 °C., and it was judged whether or not the bacteria had grown three days later. The minimum concentration in which the bacteria had grown was MIC.

Still more, antibacterial activity can be evaluated by the MIC. When the concentration of the test material is low, it does not affect the microbes. As the concentration gets higher, growth inhibition occurs. Further, as the concentration gets higher, the growth inhibition progresses, eventually to stop growth. The concentration at the final point is shown as MIC. Accordingly, when the concentration goes above MIC, microbes die.

[Result]

MIC on the each sample is shown as below.

(Table 1) The minimum concentration for Farnesol (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Farnesol	5000	78	5000	5000
50:50 by weight mixture of the two	5000	313	5000	2500
1,2-Octanediol therein	2500	156	2500	1250
Farnesol therein	2500	156	2500	1250

(Table 2) The minimum concentration for α-Bisabolol (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
α-Bisabolol	5000	625	5000	5000
50:50 weight mixture of the two	5000	1250	5000	2500
1,2-Octanediol therein	2500	625	2500	1250
α-Bisabolol	2500	625	2500	1250

(Table3) The minimum concentration for Limonene (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Limonene	5000	5000	5000	5000
50:50 weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Limonene therein	2500	2500	2500	1250

(Table 4) The minimum concentration for Camphor (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Camphor	5000	5000	5000	5000
50:50 weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Camphor therein	2500	2500	2500	1250

(Table 5) The minimum concentration for Citronella oil (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Citronella oil	1250	1250	5000	5000
50:50 weight mixture of the two	2500	2500	5000	2500
1,2-Octanediol therein	1250	1250	2500	1250
Citronella oil therein	1250	1250	2500	1250

(Table 6) The minimum concentration for Eucalyptus oil (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Eucalyptus oil	5000	5000	5000	5000
50:50 weight mixture of the two	5000	5000	5000	2500
1,2-Octanediol therein	2500	2500	2500	1250
Eucalyptus oil therein	2500	2500	2500	1250

(Table 7) The minimum concentration for Basil oil (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Basil oil	5000	5000	5000	5000
50:50 weight mixture of the two	2500	5000	5000	2500
1,2-Octanediol therein	1250	2500	2500	1250
Basil oil therein	1250	2500	2500	1250

(Table 8) The minimum concentration for Striped bamboo extract (μg/mL)

	C.albicans	S.aureus	P.aeruginosa	E.coli
1,2-Octanediol	1500	2250	3000	1250
Striped bamboo extract	5000	5000	5000	5000
50:50 weight mixture of the two	2500	5000	5000	2500
1,2-Octanediol therein	1250	2500	2500	1250
Striped bamboo extract therein	1250	2500	2500	1250

Next, the obtained each MIC of 1,2-octanediol, the samples such as perfume, essential oil or extract, and the 50:50 mixture by weight of 1,2-octanediol and the samples corresponding the compounding amount of 1,2-octanediol and the samples was plotted to make out the dual minimum inhibitory concentration diagram. Action and effect in case of that two kinds of antibacterial materials are compounded can be judged by the dual minimum inhibitory concentration diagram.

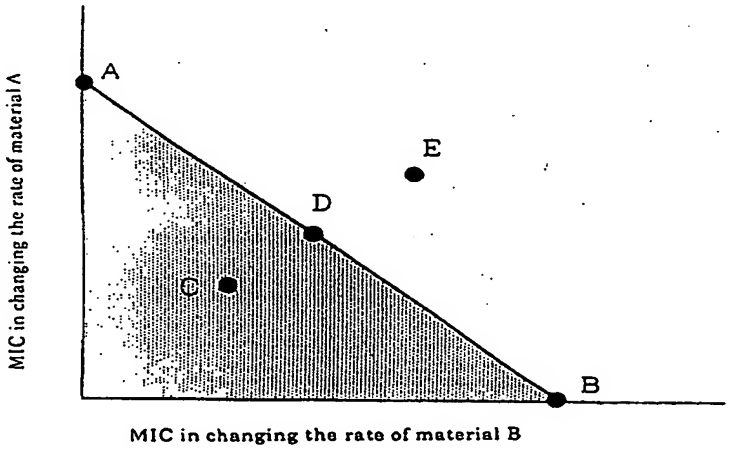
In this page, we would like to explain about evaluation of minimum inhibitory concentration (MIC) using dual minimum inhibitory concentration diagram, which is also described in the present specification.

(Evaluation of MIC)

The actions caused by compounding two antibacterial materials are roughly classified into synergistic action, additive action and counteraction. Synergistic action is the action in that two kinds of agent act synergistically, to enhance the antibacterial activity that the agents originally have. The additive action is the action in that the antibacterial activities of the agents are put together. The counteraction is the action in that one agent cancels the antibacterial activity of the other agent.

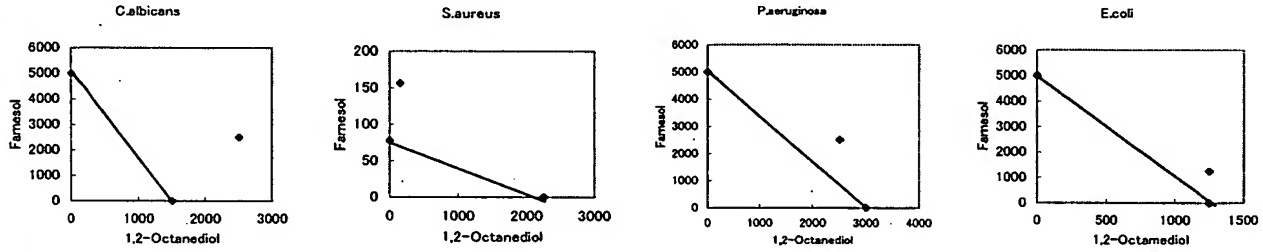
The method using dual minimum inhibitory concentration, as shown in Fig. 1-A for example, is the method to measure MICs as to material A and Material B at different proportions of them and to judge the result in view of the graph. In this method, a line is drawn to connect MIC (point A) in using only material A to MIC (point B) in using only material B. When the MIC (point C) in using both materials is inside the line, it is judged to be Synergistic action that antibacterial activity was strengthened by the combined use. When the MIC (point D) is on the line, it can be judged to be the additive action. When the MIC (point E) is in the outside of the line, it is judged to be the counteraction that cancels antibacterial activity of one or both of the materials to decrease the antibacterial activity.

(Fig 1-A) Diagram showing an example of a method to determine the effect of compounding two antibacterial materials by means of dual minimum inhibitory concentration

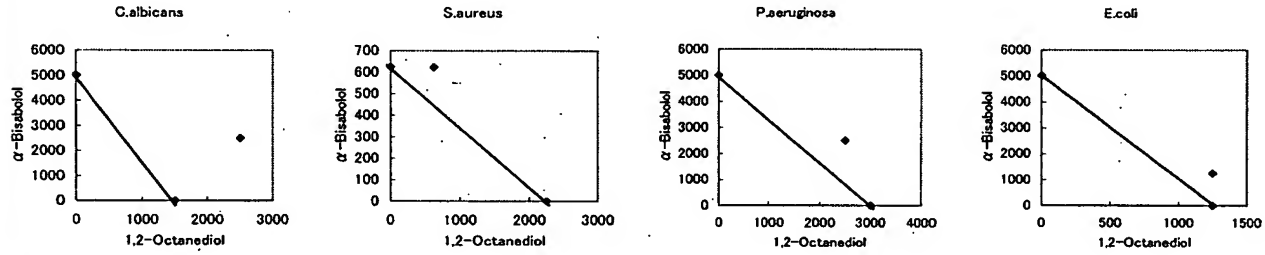


Back to the outstanding test result, the dual minimum inhibitory concentration diagram as to the each samples are shown as below. (See the Fig. 1 to Fig. 8) In the each Figs, the diagrams for C.albicans, S.aureus, P.aeruginosa and E.coli appear from left to right in that order

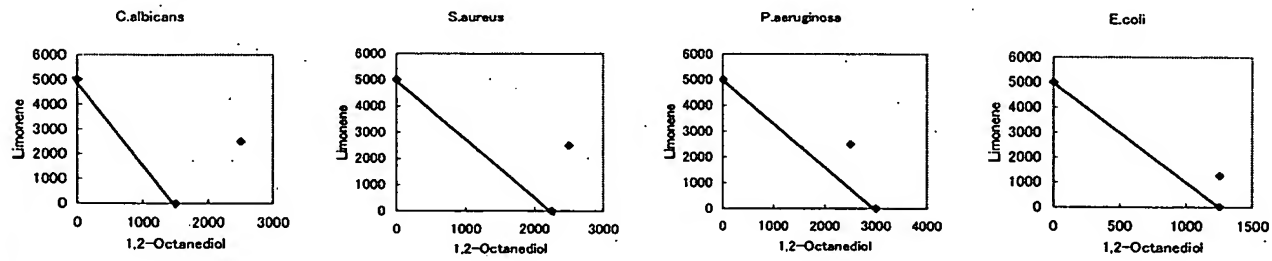
(Fig. 1) The dual minimum inhibitory concentration diagram for Farnesol



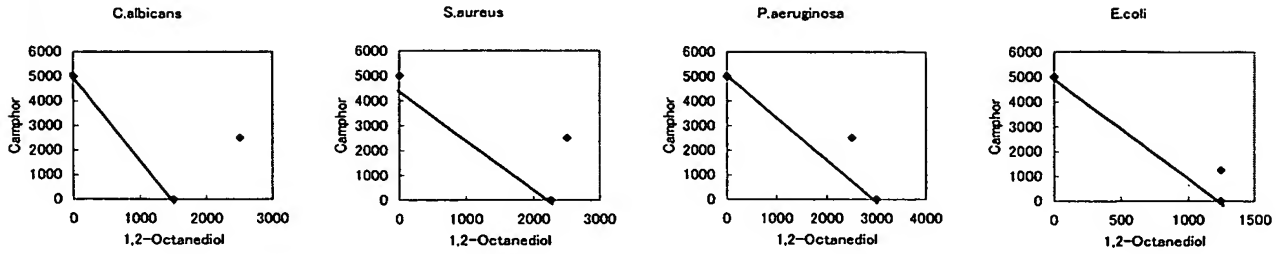
(Fig. 2) The dual minimum inhibitory concentration diagram for α-Bisabolol



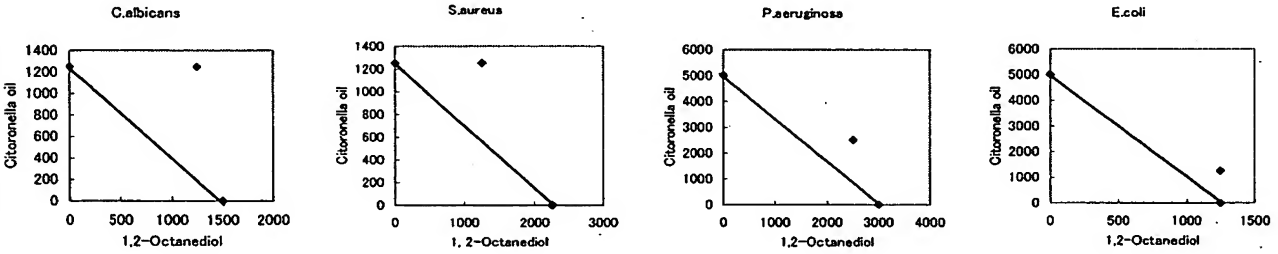
(Fig. 3) The dual minimum inhibitory concentration diagram for Limonene



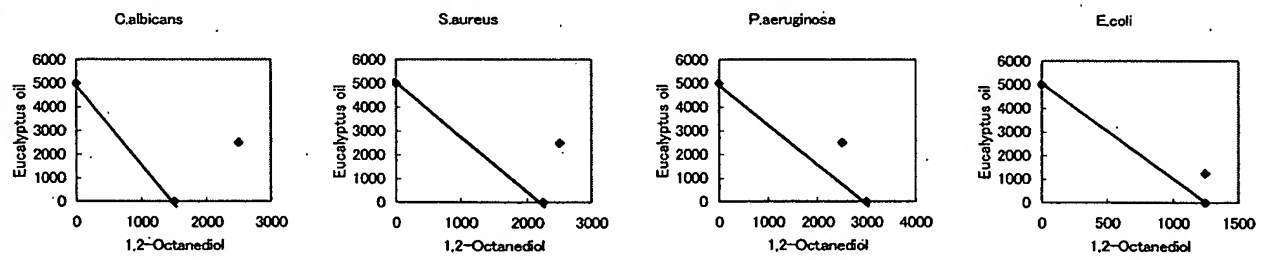
(Fig. 4) The dual minimum inhibitory concentration diagram for Camphor



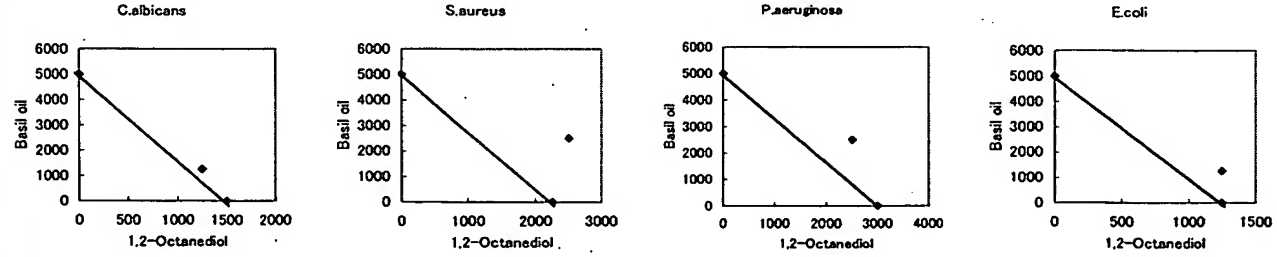
(Fig. 5) The dual minimum inhibitory concentration diagram for Citronella oil



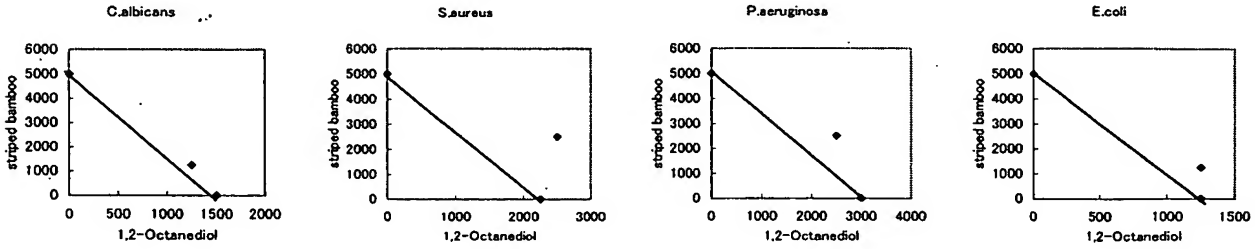
(Fig. 6) The dual minimum inhibitory concentration diagram for Eucalyptus oil



(Fig. 7) The dual minimum inhibitory concentration diagram for Basil oil



(Fig. 8) The dual minimum inhibitory concentration diagram for Striped bamboo extract



Further, according to the dual minimum inhibitory concentration diagram of Fig. 1 to Fig. 8, how antibacterial activity obtained by combination of the 1,2-octanediol and each examples is determined by the following evaluation criterion. The evaluation results are shown in the Table 10.

○: There is synergistic action in the antibacterial effect.

△: There is additive action in the antibacterial effect.

×: There is counteraction in the antibacterial effect.

(Table 10)

Samples	Fig.	C.albicans	S.aureus	P.aeruginosa	E.coli
Farnesol	1	×	×	×	×
α-Bisabolol	2	×	×	×	×
Limonene	3	×	×	×	×
Camphor	4	×	×	×	×
Citronella oil	5	×	×	×	×
Eucalyptus oil	6	×	×	×	×
Basil oil	7	△	×	×	×
Striped bamboo extract	8	△	×	×	×

[Consideration]

We selected Farnesol, α-Bisabolol, Limonene and Camphor as the sample perfume to be tested. Among them, Camphor is described in the US Patent No. 5,403,587 (McCue et al.). Further we selected Citronella oil, Eucalyptus oil, Basil oil and striped bamboo extract as the sample essential oil or extract to be tested. These are all known as having antibacterial activity, and Citronella oil and Eucalyptus oil are also described in the McCue et al.

In the results of the above-test for confirming whether the perfume such as Camphor or the like, and essential oil or extract known as antibacterial

compounds enhance the antibacterial activity that 1,2-alkanediol originally has against a broad range of strain, all tested samples show counteraction in its antibacterial effect against all tested strains when combined with 1,2-octanediol. Among them, Basil oil and striped bamboo extract showed additive action only against C.albicans, and none of the sample showed synergistic action (see the Table 10).

Finally, by comparing the examples in the present specification of this case with the test result on this declaration, we are able to conclude that only particular perfumes can enhance antibacterial activity that 1,2-alkanediol originally has, against a broad range of strains, thus only particular perfumes show synergistic action with the particular 1,2-alkanediol.

Thus, unless the particular perfumes and particular 1,2-alkanediol such as those indicated in the claims 1-12 according to the present invention are selected, synergistic action in their antibacterial effect against a broad range of the strains can not be achieved.

In conclusion, because such synergistic effect obtained from combinations of such particular perfumes and 1,2-alkanediol is unexpected, and therefore can not be readily assumed by person in the art.

6. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true.

Date: Feb. 13. 2007

Fumihiro Okada

Date: Feb. 13. 2007

Hiroya Okamoto